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The Immunizing Value of Commercial Vaccines and Bacterins Against Hemorrhagic Septicemia

L. VAN ES AND H. M. MARTIN

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THE IMMUNIZING VALUE OF COMMERCIAL VACCINES AND BACTERINS AGAINST HEMORRHAGIC SEPTICEMIA

By

L. VAN ES AND H. M. MARTIN

In the course of certain studies having to do with hemorrhagic septicemia, we were particularly struck with the difficulties which we encountered in the immunization of susceptible laboratory animals (rabbits). Aside from a rather transitory and very unstable immunity secured by the use of an anti-serum prepared by us, there was little or no evidence that injections of bacterins or vaccines, as made in our laboratory, were followed by any degree of resistance against a trial injection of *B. bipolaris*.

This difficulty in no small degree excited our curiosity regarding the immunizing value of the considerable number of "Hemorrhagic Septicemia bacterins and vaccines" which during recent years have become such a prominent article of commerce. Most of those preparations appear to be based upon a belief that by heating or by other means of killing or attenuation of the *B. bipolaris* a dependable immunizing agent can be produced, although we are not aware that this has been proven to be the case by exact laboratory experiments. In addition we have seen no evidence that the immunizing value of the products mentioned is being controlled by any standard method by either the manufacturer or the user. Considerations of this kind, however, do not appear to have a restrictive influence on the sale of those substances and we are certain that the amount of money annually expended on them by the raisers of farm animals is quite a considerable one.

In the hope of securing some information relative to the immunizing value of the agents mentioned, we undertook a series of experiments in which the immunity of the bacterin and vaccine injected laboratory animals was definitely tested by subsequent inoculations with *B. bipolaris*.

In nearly all the cases, the vaccines or bacterins were purchased directly from the manufacturer, selecting those especially which were most conspicuously advertised. The degree of immunity produced by them was always tested by the use of *B. bipolaris* cultures or material primarily obtained from the same species as the one upon which the bacterin or vaccine was supposed to confer protection. In one instance we were even fortunate enough to test the immunity of our test animals against the very strain of *B. bipolaris* found in the preparation.

In the matter of dosage, we have with but few exceptions selected the one recommended on the label. In some cases we used smaller, in others larger doses in order to ascertain a possible influence upon immunity by such variations.

In only a few instances did the vaccine or bacterin injections produce any immediate unfavorable effect on the test rabbits. In most of those cases there was indisposition or even sudden deaths immediately following the injection and this we could attribute entirely to the presence of preservative agents. With those few exceptions we encountered no trouble caused by the relative large doses.

SAMPLE No. 113.

The first preparation which we had occasion to give a trial was one sold as a "Mixed Infection Bacterin." This name, at first sight, made it appear that it would perhaps be unwise to expect an immunity against hemorrhagic septicemia from its use. However, in accompanying printed matter the manufacturer assured us that it contained forty-five per cent of *B. suis* septicus, and because of the further statement that "Each two mill contains the most satisfactory combinations of organisms and in amounts sufficient to insure immunity against each possible infection," we considered ourselves justified in using this bacterin in our tests. In passing it may be of interest to observe that according to the manufacturer's claims it should cause immunity against four other bacterial species aside from hemorrhagic septicemia.

To what extent any claims in regard to the latter disease are sustained by our experiments may be seen from Table I.

TABLE I

| Rabbit No. | Commercial mixed infection bacterin No. 113 | | Injection of one loopful of culture <i>B. bipolaris</i> No. 59 | | Date of deaths | Remarks |
|---------------|---|------------------|---|------|----------------------|-------------------------|
| | 1/7 | 1/14 | | | | |
| | Quantity c.c. | Quantity c.c. | 1/14 | 1/27 | | |
| 1..... | .5 | 2 | — | X | 1/29 | Control I Control II |
| 2..... | .5 | 2 | — | X | 1/28 | |
| 3..... | .5 | — | X | — | 1/15 | |
| 4..... | .5 | — | X | — | 1/15 | |
| 5..... | .5 | — | X | — | 1/15 | |
| 6..... | 1 | — | X | X | — | |
| 7..... | 1 | — | X | — | 1/15 | |
| 8..... | 1 | — | X | X | — | |
| 9..... | 2 | — | X | — | 1/15 | |
| 10..... | 2 | — | X | — | 1/16 | |
| 11..... | 2 | — | X | — | 1/15 | |
| 12..... | — | — | X | — | 1/15 | |
| 13..... | — | — | X | — | 1/15 | |

NOTE—The fact that an injection was made is indicated by the X. A dash (—) is used to show that no injection took place.

It will be observed that two of the three rabbits which received the 1 c.c. doses actually survived. Therefore it was thought wise to make some further tests with the same dose. What happened to the rabbits so treated is shown in Table II.

TABLE II

| Rabbit No. | Commercial mixed infection bacterin No. 113 1/17 Quantity c.c. | Injection of one loopful of culture <i>B. bipolaris</i> No. 59 1/27 | Date of deaths | Remarks |
|---------------|--|---|----------------------|---------|
| 1..... | 1 | X | 1/28 | |
| 2..... | 1 | X | 1/28 | |
| 3..... | 1 | X | 1/28 | |
| 4..... | 1 | X | 1/28 | |
| 5..... | 1 | X | 1/28 | |

SAMPLE No. 148.

The material used in this test reached the laboratory under the label: "Hemorrhagic Septicemia Vaccine, (Avian)." The detailed account of the experiment is given in Table III.

TABLE III

| Rabbit No. | Fowl cholera vaccine No. 148 2/3 Quantity 1 c.c. | One loopful culture B. bipolaris avisepticus 2/11 Strain 38 B. | Date of deaths | Remarks |
|---------------|---|---|----------------------|--|
| 1..... | — | X | 2/12 | All injections were made intravenously. |
| 2..... | — | X | 2/12 | |
| 3..... | X | X | 2/12 | |
| 4..... | X | X | 2/12 | |
| 5..... | X | X | 2/12 | |
| 6..... | X | X | 2/12 | |
| 7..... | X | X | 2/12 | |
| 8..... | X | X | 2/12 | |
| 9..... | X | X | 2/12 | |
| 10..... | X | X | 2/12 | |
| 11..... | X | X | 2/12 | |
| 12..... | X | X | 2/12 | |

SAMPLE NO. 176

A preparation sold as "Hemorrhagic Septicemia Bacterin for Cattle." The test gave the results shown in Table IV.

TABLE IV

| Rabbit No. | Commercial bacterin No. 176 2 c.c. | | Injection of one loopful of culture B. bipolaris No. 156 2/28 | Dates of deaths | Remarks |
|---------------|---|------|--|-----------------------|---|
| | 2/17 | 2/21 | | | |
| 1..... | — | — | X | 3/1 | Subject in poor condition. { Survived injection of strain 156, but when injected 3/8 with strain 59 it died two days later. |
| 2..... | — | — | X | 3/1 | |
| 3..... | X | X | X | 3/1 | |
| 4..... | X | X | X | 3/10 | Survived injection of strain 156, but when injected on 3/8 with strain 59 it died two days later. |
| 5..... | X | X | X | 3/1 | |
| 6..... | X | X | X | 3/10 | |
| 7..... | X | X | X | 3/2 | |
| 8..... | X | X | X | 3/4 | |
| 9..... | X | X | X | 3/3 | |
| 10..... | X | X | X | 3/4 | |

SAMPLE NO. 359.

This preparation was sold to us as "Hemorrhagic Septicemia Combined Bacterin (Bovine)." The immunity of the rabbits treated with this substance was tested with three strains of *B. bipolaris* of bovine origin. It will be noted that the virulence of one of those strains was lost and that the rabbits with one exception succumbed to a subsequent infection. For details of this experiment see Tables V, VI and VII.

TABLE V

| Rabbit No. | Commercial bacterin No. 359 2 c.c. 6/18 | Injection of one loopful of virulent blood B. bipolaris No. 172 7/1 | Injection of one loopful of virulent blood B. bipolaris No. 214 7/12 | Dates of deaths | Remarks |
|------------|---|---|--|-----------------|---|
| 1..... | X | X | — | 7/12 | { Subject was sick one week before death. |
| 2..... | X | X | — | 7/2 | |
| 3..... | X | X | — | 7/5 | |
| 4..... | X | X | — | 7/5 | { Sick for several days. Marked pneumonia. No organisms in heart's blood. |
| 5..... | X | X | — | 7/2 | |
| 6..... | X | X | — | 7/2 | |
| 7..... | X | X | — | 7/25 | { Sick for several days. Marked pneumonia. No organisms in heart's blood. |
| 8..... | X | X | — | 7/2 | |
| 9..... | — | X | — | 7/4 | |

TABLE VI

| Rabbit No. | Commercial bacterin No. 359 2 c.c. 6/18 | Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/1 | Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/12 | Dates of deaths | Remarks |
|------------|---|---|--|-----------------|--|
| 1..... | X | X | — | 7/2 | { Was sick for three days previous to 7/12. B. bipolaris in heart blood. |
| 2..... | X | X | — | 7/2 | |
| 3..... | X | X | — | 7/7 | |
| 4..... | X | X | — | 7/16 | { Was sick for three days previous to 7/12. B. bipolaris in heart blood. |
| 5..... | X | X | — | 7/2 | |
| 6..... | X | X | — | 7/2 | |
| 7..... | X | X | — | 7/2 | { Was sick for three days previous to 7/12. B. bipolaris in heart blood. |
| 8..... | X | X | — | 7/2 | |
| 9..... | X | X | — | 7/2 | |
| 10..... | X | X | — | 7/2 | { Was sick for three days previous to 7/12. B. bipolaris in heart blood. |
| 11..... | X | X | — | 7/8 | |

TABLE VII

| Rabbit No. | Commercial bacterin No. 359 2 c.c. 6/18 | Injection of one loopful of virulent blood (B. bipolaris) No. 307 7/1 | Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/12 | Dates of deaths | Remarks |
|------------|---|---|--|-----------------|--|
| 1..... | X | X | X | 7/13 | { Pleuro pneumonia B. bipolaris in blood. |
| 2..... | X | X | X | — | |
| 3..... | X | X | X | 7/7 | |
| 4..... | X | X | — | 7/8 | |
| 5..... | X | X | — | 7/22 | |
| 6..... | X | X | X | 7/13 | |
| 7..... | X | X | X | 7/13 | |
| 8..... | X | X | — | 7/4 | |
| 9..... | X | X | X | 7/13 | |
| 10..... | X | X | — | 7/3 | |
| 11..... | — | X | X | 7/13 | |
| 12..... | — | X | X | 7/13 | |

With the same combined bacterin we furthermore treated three rabbits not less than eight times at forty-eight hour intervals. The results are given in Table VIII.

TABLE VIII

| Rabbit No. | Received 2 c.c. of commercial bacterin No. 359 on the following dates: | | Injection of one loopful of virulent blood (B. bipolaris) No. 214 7/9 | Injection of one loopful of virulent blood (B. bipolaris) No. 172 7/12 | Dates of deaths | Remarks |
|------------|--|------|---|--|-----------------|---------|
| | 6/18 | 6/28 | | | | |
| | 6/20 | 6/30 | | | | |
| | 6/23 | 7/1 | | | | |
| | 6/27 | 7/3 | | | | |
| 1..... | X | | X | X | 7/13 | |
| 2..... | X | | X | — | 7/10 | |
| 3..... | X | | X | — | 7/10 | |
| 4..... | — | | X | — | 7/10 | |
| 5..... | — | | X | — | 7/10 | |

SAMPLE No. 485.

A product sold as "Bacillus Suisepcticus Bacterin (for swine plague)."

As the manufacturer of this material in common with others, indicates in his advertising matter that repeated bacterin injections are desirable, we designed this series of experiments so that three groups of six rabbits each received respectively one, two and three bacterin injections while another group of ten rabbits were injected not less than twelve times. All injections were made every other day and were so timed that the entire series received its last immunizing dose on the same day.

Ten days later each of the rabbits in this series received an inoculation with 1/10 c.c. of virus mixture prepared by taking the heart blood of a rabbit dead as a result of inoculation with *B. bipolaris* (from swine) mixed with twenty parts of salt solution and added to equal parts of a 24-hour boullion culture of the same organism. Table IX shows the results of those trials.

TABLE IX

| Rabbit No. | No. 485 Bacterin injections 2 c.c. | | | | | | | | | | | Injections B. bipolaris No. 49 1-10 c.c. 10-25 | Dates of deaths | Remarks |
|---------------|------------------------------------|------|------|------|------|------|------|------|------|-------|-------|--|-----------------------|--|
| | 9-23 | 9-25 | 9-27 | 9-29 | 10-1 | 10-3 | 10-5 | 10-7 | 10-9 | 10-11 | 10-13 | 10-15 | | |
| 1..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | { Died 2½ hours after injection. B. bipolaris in heart blood. |
| 2..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 3..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 4..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 5..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 6..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 7..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 8..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 9..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 11..... | X | X | X | X | X | X | X | X | X | X | X | X | 10/26 | |
| 12..... | — | — | — | — | — | — | — | — | — | X | X | X | 10/25 | { Died 2½ hours after injection. B. bipolaris in heart blood. |
| 13..... | — | — | — | — | — | — | — | — | — | X | X | X | 10/26 | |
| 14..... | — | — | — | — | — | — | — | — | — | X | X | X | 10/26 | |
| 15..... | — | — | — | — | — | — | — | — | — | X | X | X | 10/26 | |
| 16..... | — | — | — | — | — | — | — | — | — | X | X | X | 10/26 | |
| 17..... | — | — | — | — | — | — | — | — | — | X | X | X | 10/26 | |
| 18..... | — | — | — | — | — | — | — | — | — | — | X | X | 10/26 | |
| 19..... | — | — | — | — | — | — | — | — | — | — | X | X | 10/25 | { Died 2½ hours after injection. |
| 20..... | — | — | — | — | — | — | — | — | — | — | X | X | 10/26 | |
| 21..... | — | — | — | — | — | — | — | — | — | — | X | X | 10/26 | |
| 22..... | — | — | — | — | — | — | — | — | — | — | X | X | 10/26 | |
| 23..... | — | — | — | — | — | — | — | — | — | — | X | X | 10/26 | |
| 24..... | — | — | — | — | — | — | — | — | — | — | — | X | 10/25 | { Died 2½ hours after injection. |
| 25..... | — | — | — | — | — | — | — | — | — | — | — | X | 10/26 | |
| 26..... | — | — | — | — | — | — | — | — | — | — | — | X | 10/26 | |
| 27..... | — | — | — | — | — | — | — | — | — | — | — | X | 10/26 | |
| 28..... | — | — | — | — | — | — | — | — | — | — | — | X | 10/26 | |
| 29..... | — | — | — | — | — | — | — | — | — | — | — | X | 10/26 | |
| 30..... | — | — | — | — | — | — | — | — | — | — | — | — | 10/26 | |
| 31..... | — | — | — | — | — | — | — | — | — | — | — | — | 10/26 | |
| 32..... | — | — | — | — | — | — | — | — | — | — | — | — | 10/25 | { Dead 2½ hours after injection. |
| 33..... | — | — | — | — | — | — | — | — | — | — | — | — | 10/26 | |

SAMPLE No. 549.

The material tested under this number varies from others tested in as much as two distinct injections are required. The first with killed organisms and the second with living ones. As the latter were virulent to rabbits, in spite of the previous injections, there was no opportunity of testing any immunity which may be obtained by this method of treatment. In the final tabulation of our results the data of Table X were not used.

TABLE X

| Rabbit No. | Bacterin No. 54.9 Injection 4 c.c 11/25 | Vaccine No. 549 Injection 4 c.c. 12/5 | Injection B. bipolaris No. 549 | Dates of deaths | Remarks |
|------------|---|---|--------------------------------------|--------------------------|---|
| 1. | X | X | — | 12/6 | |
| 2. | X | X | — | 12/6 | |
| 3. | X | X | — | 12/6 | |
| 4. | X | X | — | 12/6 | |
| 5. | X | X | — | 12/5 | |
| 6. | X | X | 12/16 | { Killed } { 1/1/20 } | { Dead four hours after vaccine injection. When killed subject had large abscesses in flank and hind quarter. |
| 7. | X | X | — | 12/6 | |
| 8. | X | X | — | 12/6 | |
| 9. | X | X | — | 12/6 | |
| 10. | X | X | — | 12/6 | |
| 11. | X | X | — | 12/6 | |
| 12. | X | X | — | 12/6 | |
| 13. | X | X | — | 12/6 | |
| 14. | X | X | — | 12/7 | |
| 15. | X | X | — | 12/6 | |
| 16. | X | X | — | 12/6 | |
| 17. | X | X | — | 12/6 | |
| 18. | X | X | — | 12/6 | |
| 19. | X | X | — | 12/6 | |
| 20. | (X) | X | — | 12/6 | (X) Only 2 c.c. of bacterin. |
| 21. | — | X | 12/15 | — | Control survived. |
| 22. | — | — | 12/15 | 12/15 | { Control. Heart blood swarming with B. bipolaris. |
| 23. | — | — | 12/16 | 12/16 | Control. Dead six hours after injection. |
| 24. | — | — | 12/16 | 12/16 | Control. Dead six hours after injection. |

SAMPLE No. 620.

A "Hemorrhagic Septicemia Vaccine for cattle." An interesting feature of this experiment is the fact that this vaccine was virulent to many rabbits of the series as eight died after the first injection and four after the second one. From those rabbits we isolated the *B. bipolaris* and this was used to test the immunity of the survivors. Of the latter several had shown sickness as a result of the vaccine injected. Table XI shows, however, that even this failed to protect them against the very virus from which the vaccine was made. The table further shows that one of those surviving rabbits (26) which had received and survived a 10 c.c. injection succumbed to subsequent infection with the organism isolated from the vaccine.

TABLE XI

| Rabbit No. | Injection vaccine No. 620 2/9 | Injection vaccine No. 620 2/14 | Injection vaccine No. 620 2/28 | Injection B. bipolaris No. 620 | Injection B. bipolaris No. 620 | Dates of deaths | Remarks |
|------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-----------------|---|
| 1..... | 2 c.c. | — | — | — | — | 2/11 | { Subject died a few hours after inoculation. Heart blood was swarming with <i>B. bipolaris</i> . |
| 2..... | 2 c.c. | 2 c.c. | — | 2/21 | — | 2/22 | |
| 3..... | 2 c.c. | 2 c.c. | — | — | — | 2/14 | |
| 4..... | 2 c.c. | — | — | — | — | 2/14 | |
| 5..... | 2 c.c. | 2 c.c. | — | — | — | 2/16 | Heart blood swarming with <i>B. bipolaris</i> . |
| 6..... | 2 c.c. | — | — | — | — | 2/12 | Heart blood swarming with <i>B. bipolaris</i> . |
| 7..... | 2 c.c. | 2 c.c. | — | 2/21 | — | 2/22 | { Heart blood swarming with <i>B. bipolaris</i> . |
| 8..... | 2 c.c. | 2 c.c. | — | 2/21 | — | 2/22 | |
| 9..... | 2 c.c. | 2 c.c. | — | 2/21 | — | 2/22 | |
| 10..... | 2 c.c. | — | — | — | — | 2/11 | |
| 11..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | Heart blood swarming with <i>B. bipolaris</i> . |
| 12..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | |
| 13..... | 4 c.c. | — | — | — | — | 2/13 | |
| 14..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | |
| 15..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | { Heart blood swarming with <i>B. bipolaris</i> . |
| 16..... | 4 c.c. | 4 c.c. | — | — | — | 2/17 | |

TABLE XI—Continued

| Rabbit No. | Injection vaccine No. 620 2/9 | Injection vaccine No. 620 2/14 | Injection vaccine No. 620 2/28 | Injection B. bipolaris No. 620 | Injection B. bipolaris No. 620 | Dates of deaths | Remarks |
|------------|-------------------------------|--------------------------------|--------------------------------|--------------------------------|--------------------------------|-----------------|--|
| 17..... | 4 c.c. | — | — | — | — | 2/14 | { Heart blood swarming with B. bipolaris. |
| 18..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | |
| 19..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | |
| 20..... | 4 c.c. | 4 c.c. | — | 2/21 | — | 2/22 | { Heart blood swarming with B. bipolaris. |
| 21..... | 1 c.c. | 1 c.c. | — | — | — | 2/16 | |
| 22..... | 1 c.c. | — | — | — | — | 2/11 | |
| 23..... | 1 c.c. | — | — | — | — | 2/14 | { Subject drowsy after last injection. Heart B. swarming with B. bipolaris. Developed apparent anaphylactic symptoms after last injection from which it recovered in 15 min. Heart blood swarming with B. bipolaris. Slight disturbance after injection of 2/28. Purulent pericarditis on which abundance of B. bipolaris. |
| 24..... | 1 c.c. | 1 c.c. | 10 c.c. | — | — | 2/29 | |
| 25..... | 1 c.c. | 1 c.c. | 10 c.c. | — | — | 2/29 | |
| 26..... | 1 c.c. | 1 c.c. | 10 c.c. | 3/9 | 3/11 | 3/15 | { Control |
| 27..... | — | — | — | 2/21 | — | 2/22 | |
| 28..... | — | — | — | 2/21 | — | 2/22 | |
| 29..... | — | — | — | 2/21 | — | 2/22 | |
| 30..... | — | — | — | 2/21 | — | 2/22 | |

SAMPLE No. 621.

This preparation was called on the label "Hemorrhagic Septicemia Vaccine for Swine." The results of our tests are shown in Table XII.

TABLE XII

| Rabbit No. | Vaccine injection No. 621 | | | Injection B. bipolaris No. 59 | Dates of deaths | Remarks |
|---------------|------------------------------|--------|---------|-------------------------------------|-----------------------|---|
| | 2/10 | 2/15 | 3/1 | | | |
| 1..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 2..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 3..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 4..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 5..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 6..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 7..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 8..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 9..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 10..... | 2 c.c. | 2 c.c. | — | 2/24 | 2/25 | |
| 11..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 12..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 13..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 14..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 15..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 16..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 17..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/24 | |
| 18..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 19..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 20..... | 4 c.c. | 4 c.c. | — | 2/24 | 2/25 | |
| 21..... | 1 c.c. | 1 c.c. | 10 c.c. | 3/9 | 3/11 | { Heart blood contained many B. bipolaris. |
| 22..... | — | — | — | 2/24 | 2/25 | Control |
| 23..... | — | — | — | 2/24 | 2/24 | Control |
| 24..... | — | — | — | 2/24 | 2/25 | Control |
| 25..... | — | — | — | 2/24 | 2/25 | Control |

SAMPLE No. 717.

A specimen of "Hemorrhagic Septicemia Bacterin (Bovine)." The details of the tests made with this material are given in Table XIII.

TABLE XIII

| Rabbit No. | Injections bacterin No. 717 5 c.c. | | | | Injections B. bipolaris No. 738 Loopful 4/19 | Dates of deaths | Remarks |
|---------------|---------------------------------------|-----|-----|-----|--|-----------------------|---------|
| | 3/31 | 4/3 | 4/6 | 4/9 | | | |
| 1..... | X | X | X | X | 1/10 | 4/20 | |
| 2..... | X | X | X | X | 1/10 | 4/20 | |
| 3..... | X | X | X | X | 1/10 | 4/20 | |
| 4..... | X | X | X | X | 1/4 | 4/20 | |
| 5..... | X | X | X | X | 1/4 | 4/20 | |
| 6..... | X | X | X | X | 1/4 | 4/20 | |
| 7..... | X | X | X | X | 1/4 | 4/20 | |
| 8..... | X | X | X | X | 1/4 | 4/20 | |
| 9..... | X | X | X | X | 1/4 | 4/20 | |
| 10..... | X | X | X | X | 1/4 | 4/20 | |
| 11..... | X | X | X | X | 1/4 | 4/20 | |
| 12..... | X | X | X | X | 1/4 | 4/20 | |
| 13..... | X | X | X | X | 1/4 | 4/20 | |
| 14..... | X | X | X | X | 1/4 | 4/20 | |
| 15..... | X | X | X | X | 1/4 | 4/20 | |
| 16..... | X | X | X | X | 1/4 | 4/20 | |
| 17..... | X | X | X | X | 1/4 | 4/20 | |
| 18..... | X | X | X | X | 1/4 | 4/20 | |
| 19..... | X | X | X | X | 1/4 | 4/20 | |
| 20..... | X | X | X | X | 1/4 | 4/20 | |
| 21..... | — | — | — | — | 1/10 | 4/20 | Control |
| 22..... | — | — | — | — | 1/4 | 4/20 | Control |

SAMPLE No. 720.

This preparation is sold as "Hemorrhagic Septicemia Vaccine (Bovine)." The experiments made with this substance are given in Table XIV.

TABLE XIV

| Rabbit No. | Injections vaccine No. 720 4 c.c. | | | Injections <i>B. bipolaris</i> No. 738 Loopful 4/19 | Dates of deaths | Remarks |
|---------------|---|-----|-----|---|-----------------------|--|
| | 4/3 | 4/6 | 4/9 | | | |
| 1..... | X | X | X | 1/10 | 4/20 | { Received more than 4 c.c. vaccine 4/3 |
| 2..... | X | X | X | 1/10 | 4/20 | |
| 3..... | X | X | X | 1/10 | 4/20 | |
| 4..... | X | X | X | 1/4 | 4/20 | |
| 5..... | X | X | X | 1/4 | 4/20 | |
| 6..... | X | X | X | 1/4 | 4/20 | |
| 7..... | X | X | X | 1/4 | 4/20 | |
| 8..... | X | X | X | 1/4 | 4/20 | |
| 9..... | X | X | X | 1/4 | 4/20 | |
| 10..... | X | X | X | 1/4 | 4/20 | |
| 11..... | X | X | X | 1/4 | 4/20 | Control Control |
| 12..... | X | X | X | 1/4 | 4/20 | |
| 14..... | X | X | X | 1/4 | 4/20 | |
| 15..... | X | X | X | 1/4 | 4/20 | |
| 16..... | X | X | X | 1/4 | 4/20 | |
| 17..... | X | X | X | 1/4 | 4/20 | |
| 18..... | X | X | X | 1/4 | 4/20 | |
| 19..... | X | X | X | 1/10 | 4/20 | |
| 20..... | X | X | X | 1/4 | 4/20 | |

SAMPLE No. 735.

This material was sold to us under the label "Swine Plague Bacterin." Table XV relates the tests made in detail.

TABLE XV

| Rabbit No. | Injections bacterin No. 735 2 c.c. | | | Injections B. bipolaris No. 59 Loopful | | Dates of deaths | Remarks |
|---------------|--|------|------|---|------|-----------------------|---|
| | 4/13 | 4/16 | 4/20 | 4/28 | 5/11 | | |
| 1..... | X | X | X | 1/50 | 1/10 | 5/12 | { Pneumonia, pericar- ditis. Organs con- tained B. bipolaris. |
| 2..... | X | X | X | 1/50 | — | 4/29 | |
| 3..... | X | X | X | 1/50 | — | 4/29 | |
| 4..... | X | X | X | 1/50 | — | 4/29 | |
| 5..... | X | X | X | 1/50 | — | 4/29 | |
| 6..... | X | X | X | 1/50 | — | 4/29 | |
| 7..... | X | X | X | 1/50 | — | 4/29 | |
| 8..... | X | X | X | 1/40 | — | 4/29 | |
| 9..... | X | X | X | 1/50 | — | 4/29 | |
| 10..... | X | X | X | 1/50 | — | 4/30 | |
| 11..... | X | X | X | 1/50 | — | 4/30 | |
| 12..... | X | X | X | 1/50 | — | 4/29 | |
| 13..... | X | X | X | 1/50 | — | 4/29 | |
| 14..... | X | X | X | 1/50 | — | 4/29 | |
| 15..... | X | X | X | 1/50 | — | 4/29 | |
| 16..... | X | X | X | 1/50 | 1/10 | 5/19 | { Control Control |
| 17..... | X | X | X | 1/50 | — | 4/29 | |
| 18..... | X | X | X | 1/50 | — | 4/29 | |
| 19..... | X | X | X | 1/50 | — | 4/29 | |
| 20..... | X | X | X | 1/50 | — | 4/29 | |
| 21..... | — | — | — | 1/50 | — | 4/29 | |
| 22..... | — | — | — | 1/50 | — | 4/29 | |

The results of the entire series of experiments as far as the acquisition of immunity on the part of the laboratory animals is concerned are summarized in Table XVI.

TABLE XVI

| No. rabbits treated and immunity tested | No. of bacterin or vaccine injections | Results | | Remarks |
|---|---------------------------------------|--------------|---------------|---|
| | | Rabbits dead | Rabbits alive | |
| 59..... | 1 | 56 | 3 | Only those rabbits which lived long enough to receive the final virus dose were considered in the construction of this table. |
| 47..... | 2 | 47 | 0 | |
| 48..... | 3 | 48 | 0 | |
| 20..... | 4 | 20 | 0 | |
| 3..... | 8 | 3 | 0 | |
| 10..... | 12 | 10 | 0 | |
| 187..... | .. | 184 | 3 | |

CONCLUSIONS

It does not appear that the vaccines and bacterins experimented with have any antigenic value which expresses itself in actual resistance to *B. bipolaris* infection. We encountered uniform negative results, no matter if we injected those substances once or a dozen times; a virus injection was always certain to kill the animals.

The indications are that if hemorrhagic septicemia is in reality a frequent and very fatal disease to our farm animals, that the commercial immunizing agents against it are indeed a poor staff to lean on and that if those diseases are at all to be combatted in a serious manner, we will have to look to other methods for relief.

